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Institute Report No. 401

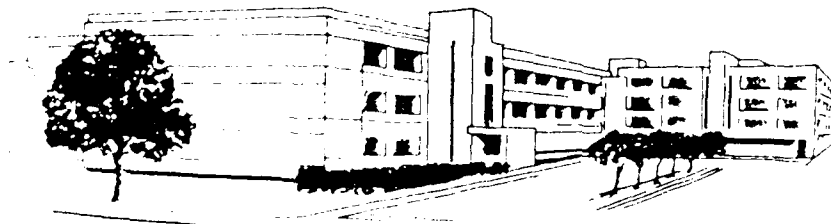
**The Effects of Hypertonic Saline (7.5%)/Dextran-70
(HSD) on Human Red Cell Typing, Lysis, and Metabolism
in Vitro**

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DIVISION OF MILITARY TRAUMA
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DIVISION OF BLOOD RESEARCH

September 1989

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REPORT DOCUMENTATION PAGE

Form Approved
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2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release, distribution is unlimited.		
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE					
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No. 401			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION Div of Military Trauma and Div of Blood Research		6b. OFFICE SYMBOL (If applicable) SGRD-ULT-M		7a. NAME OF MONITORING ORGANIZATION U.S. Army Medical Research and Development Command	
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800		7b. ADDRESS (City, State, and ZIP Code) Ft. Detrick Frederick, MD 21701-5012			
8a. NAME OF FUNDING / SPONSORING ORGANIZATION		8b. OFFICE SYMBOL (If applicable)		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c. ADDRESS (City, State, and ZIP Code)		10. SOURCE OF FUNDING NUMBERS			
		PROGRAM ELEMENT NO. 63807A		PROJECT NO. 807D836	TASK NO. AX WORK UNIT 087
11. TITLE (Include Security Classification) (U) The Effects of Hypertonic Saline (7.5%)/Dextran-70 (HSD) on Human Red Cell Typing, Lysis, and Metabolism in vitro.					
12. PERSONAL AUTHOR(S) GL Moore, JJ Summary, MA Dubick, ME Ledford, BA Ryan, A Gonzales, and CE Wade.					
13a. TYPE OF REPORT Institute		13b. TIME COVERED FROM _____ TO _____		14. DATE OF REPORT (Year, Month, Day) August 1989	
15. PAGE COUNT 12					
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP	(U) Hypertonic Saline, Dextran-70, Red Cell Typing, Red Cell Lysis, Blood Storage (U)		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) <p>The introduction of a 7.5% hypertonic saline/6% Dextran-70 (HSD) solution into clinical trials for the treatment of hypovolemic states, and the past concerns regarding possible interference of dextran with blood serology, prompted us to investigate the effects of HSD on human red cell typing and stability. HSD was evaluated with fresh and 35-day stored CPDA-1 red cells from 12 healthy donors. A 1:5 mixture of HSD to blood had no effect on ABO, Rh, and MN typing in both fresh and stored blood. HSD produced no significant lysis with fresh cells and a minimal level with stored blood. No evidence of metabolic or morphologic changes were seen after HSD treatment. The results of this study suggest that clinical use of HSD for treatment of hemorrhagic shock will not affect blood group determinations or red cell stability from stored blood which may be infused after the HSD treated patient is transported to a hospital.</p>					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED		
22a. NAME OF RESPONSIBLE INDIVIDUAL William C. Cole, COL, VC, Acting Commander			22b. TELEPHONE (Include Area Code) (415) 561-5816		22c. OFFICE SYMBOL SGRD-ULT-M

ABSTRACT

The introduction of a 7.5% hypertonic saline/6% Dextran-70 (HSD) solution into clinical trials for the treatment of hypovolemic states, and the past concerns regarding possible interference of dextran with blood serology, prompted us to investigate the effects of HSD on human red cell typing and stability. HSD was evaluated with fresh and 35-day stored CPDA-1 red cells from 12 healthy donors. A 1:5 mixture of HSD to blood had no effect on ABO, Rh, and MN typing in both fresh and stored blood. HSD produced no significant lysis with fresh cells and a minimal level with stored blood. No evidence of metabolic or morphologic changes were seen after HSD treatment. The results of this study suggest that clinical use of HSD for treatment of hemorrhagic shock will not affect blood group determinations or red cell stability from stored blood which may be infused after the HSD treated patient is transported to a hospital.

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The Effects of Hypertonic Saline(7.5%)/Dextran-70 (HSD) on Human Red Cell Typing, Lysis, and Metabolism in vitro. -- Moore et al.

INTRODUCTION

In trauma scenarios initial treatment of hemorrhage and shock is frequently done with crystalloid solutions. Present medical doctrine calls for three volumes of saline or Ringer's solution per estimated volume of blood lost. More recently a 7.5% hypertonic saline / 6% Dextran-70 solution (HSD) has been shown effective in reducing volume requirements from 3 to 0.25 volumes for treatment of hypoxia (1,2). Preliminary studies in human trauma patients suggest that it may increase survival (3,4).

Previous experience with dextran has indicated that it may interfere with the ability to type and crossmatch red cells (5-7), presumably through its induction of rouleaux formation (7). Effects of dextran on typing or crossmatching are a direct function of the dextran molecular weight and the quality of the stored red cells (6). Many of the adverse effects are associated with dextran average molecular weights above 150,000 (9,10). This could be a problem for the HSD resuscitated patient who subsequently is transfused in the emergency room.

An additional problem has been the in vitro observation of increasing lysis when dog red cells were exposed to doses of hypertonic saline solutions above 7.5% (8). While it is well known that dog red cells are more fragile than human, and readily lyse under slight stress, stored human blood is also well known to increase its fragility during banked storage.

In this study we have examined the effects of HSD on both fresh and 35 day stored human red cells to determine if HSD altered the cell's metabolism, degree of lysis, or ability to be typed.

METHODS

Units of whole blood (450 ml each) were drawn with informed consent from twelve healthy adult volunteers. The blood was collected into Fenwal CPDA-1 bags according to American Association of Blood Banks Standards (11). Volunteers were chosen to insure that all ABO blood types were represented. Forty ml of blood was removed for day zero analysis, and the remainder of the blood was stored at 3°C for 35 days, then re-analysed.

On day zero and day 35 the 40 ml aliquot of blood from each donor was divided into two 20 ml volumes in centrifuge tubes. To

one tube was added 5 ml of isotonic saline containing 200 mg/dl of glucose (CTR). To the other paired tube was added 5 ml of the HSD test solution (HSD), which was prepared by Pharmacia AB, Uppsala, Sweden. Both tubes were capped, mixed, and incubated for 30 min. in a 37°C water bath with remixing at 10 min. intervals. The tubes were then centrifuged at 3400xg for 10 min. The supernatant was removed and saved for hemoglobin analysis. The packed cells were resuspended in 25 ml of isotonic saline plus glucose and centrifuged to wash out residual hypertonic saline and to return the cells to normal volume. These washed cells were then suspended in an equal volume of isotonic saline and evaluated. The mixing ratio of 1:5 was chosen as a worse-case situation. A typical 70 kg man has a blood volume of about 5000 ml. If half of this volume were lost, then resuscitation would be done with 250 ml of HSD, for a 1:10 ratio. Smaller people and larger blood loss might reduce the ratio further, but 1:5 is the maximum ratio conceived for surviving patients.

During evaluation the cells were typed (11) and assayed for osmotic fragility (12), morphology (13), ATP (14), and 2,3-DPG concentrations (14). The saline wash solution was saved for hemoglobin analysis (15). All bags were cultured at the end of the study to insure that they had remained sterile. Blood typing for ABO, D(Rho) and MN antigens were performed using commercial (American Hospital Supply, Miami, FL) antisera and controls. Agglutination results were scored on the basis of no agglutination (N) to (+4). Statistical comparisons were made using the paired t-test in BMDP 3D. A 0.05 level of probability was used.

RESULTS

Typical osmotic fragility curves for one donor are shown in Figure 1. HSD treatment of red cells causes a slight right shift in the curve which is statistically but not clinically significant. The osmotic fragility data was evaluated by linear regression of the linear, mid portion, of the curve (0.425 to 0.525 percent saline) and testing for differences in the 50% lysis salt concentration, the slope of the regression, and the correlation coefficient (16). These data are summarized in Table 1. Statistical differences were seen between CTR and HSD at both time periods and between time periods with both CTR and HSD 50% lysis means. Similar statistical differences were seen between the slopes of the regression lines, but no significant differences were seen among the correlation coefficients.

Red cell lysis was monitored by measuring both the supernatant hemoglobin levels following 37°C incubation of the cells, and the isotonic saline wash solution subsequent to

incubation. The data are summarized in Figure 2. There were no significant differences between the mean supernatant values at Day 0. Significant differences (control vs HSD) were seen between Day 0. washes, Day 35 supernatants, and Day 35 wash means.

Figure 3 shows the mean values for red cell morphology index, ATP and 2,3-DPG. There were no significant differences between CTR and HSD groups. The control results were typical of previous storage studies in CPDA-1 (17).

No pseudoagglutination or rouleaux formation occurred in the blood of the 12 donors typed in this study, resulting in no ABO, D, or MN discrepancies in the fresh or stored red cells following HSD treatment. The strength of the agglutinations (Table 2) were identical, except for one case.

DISCUSSION

In over 40 years of clinical use, primarily as a volume expander in the management of shock, dextran was occasionally observed to effect the behavior of red blood cells (7,9,18). Depending on the average molecular weight of the preparation, dextrans could decrease as well as increase red cell aggregation, even to the extent of promoting rouleaux formation (9,18,19). Such concerns about possible pseudoagglutination induced by dextran prompted a number of investigators to evaluate the effects of dextran on typing and crossmatching of blood. Following an extensive review of the literature, Gruber (9) reported that interference with red cell serology was observed only with large molecular weight (>150,000 dalton) dextrans and not with dextrans of 40-70,000 daltons. The present study, which uses Dextran of 70,000 daltons in the HSD, shows no effect on red cell typing and thus confirms the earlier efforts. In addition we have previously observed that infusion of HSD at therapeutic doses to euvoletic or hemorrhaged rabbits and pigs (4) does not induce significant rouleaux formation (20), suggesting that HSD infusion would not effect red cell typing or crossmatching. The hypertonic saline component of HSD also does not effect typing which is consistent with the observation that red cell typing or crossmatching is not affected by intravenous infusion of electrolyte solutions (21). This is also shown in the deglycerolization of frozen-thawed red cells which are treated with 12% saline prior to washing with isotonic saline (22). The final washed cells show no loss in ability to be typed or crossmatched.

Red cells are good osmometers with great resistance to osmotic lysis. Treatment of thawed glycerolized cells with 12% saline lyses only a small fraction of 1% of the cells (22). This

resistance to lysis was also shown by Rocha and Silva for dog red cells which did not hemolyse until saline concentrations exceeded 15% (8). The Day-0 arm of our study confirmed this resistance to lysis with fresh CPD banked blood. These cells did shrink in the presence of HSD and were somewhat difficult to resuspend in isotonic saline, but did not show evidence of rouleaux formation. When resuspended in isotonic saline the cells returned to normal suspension behavior and had normal size and morphology. HSD treatment did not alter the levels of ATP or 2,3-DPG in the cells, implying that this treatment did not affect red cell metabolism by causing egress of metabolites, salt inactivation of glycolytic enzymes, or general leakiness of the membranes. Stored red cells are more prone to lysis than fresh cells, as observed in our study between fresh and 35 day controls. HSD did cause a significant increase in lysis of the 35 day stored red cells when compared to controls. However, this lysis, which could equal 2 or 3 percent of the cells, may not represent an increased decrement of therapeutic red cells because the 35 day cells would only have a 24 hour survivability of about 75 %. Thus the cells lysed by HSD may be the oldest cells which would lyse or be cleared anyway upon infusion. It is interesting that all of the HSD-induced lysis we saw occurred in the wash, not when HSD was added to the cells. This suggests that lytic damage is a function of excess salt leaving the red cells, but not entering them.

The MN antigen system was measured because it is attached to the membrane protein glycophorin A. This protein is involved with protein 4.1 and band 3 protein in a mechanism, not yet understood, which seems to help bind together the major structural proteins in the membrane skeleton (23). Change in ability to measure MN, which was not seen in our study, may be associated with loss of membrane integrity.

The effects of HSD on fresh or stored banked red cells seems to be limited to a slight amount of lysis after exposure to HSD and during return to isotonicity. This effect becomes somewhat more pronounced as the blood ages and becomes more fragile, but is not of sufficient magnitude cause concern. No changes in ability to measure red cell antigens was observed. Therefore, at the doses used to treat hemorrhagic shock, it does not appear that HSD poses a significant clinical problem with respect to cell typing or the stability of banked blood.

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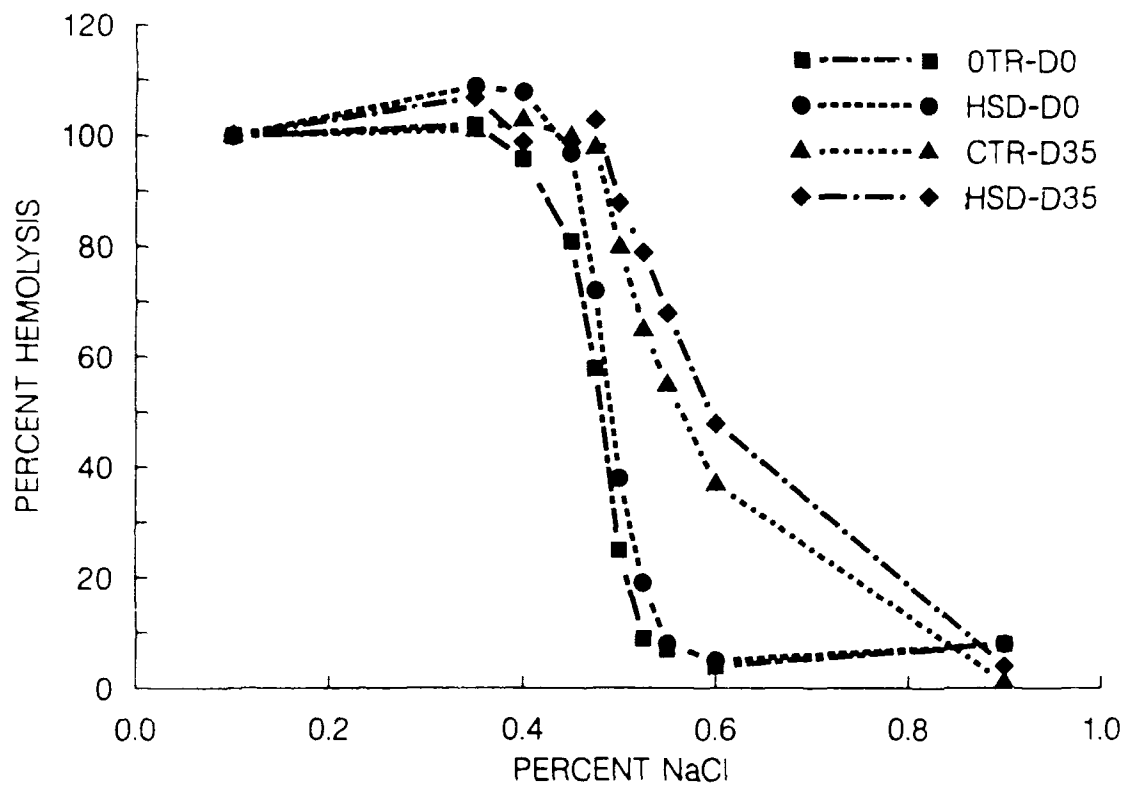


Figure 1: Typical osmotic fragility curve shown for one donor in the study. Statistical data calculated on the linear drop portion of the curve between salt % of 0.425 and 0.5, or 0.45 and 0.60.

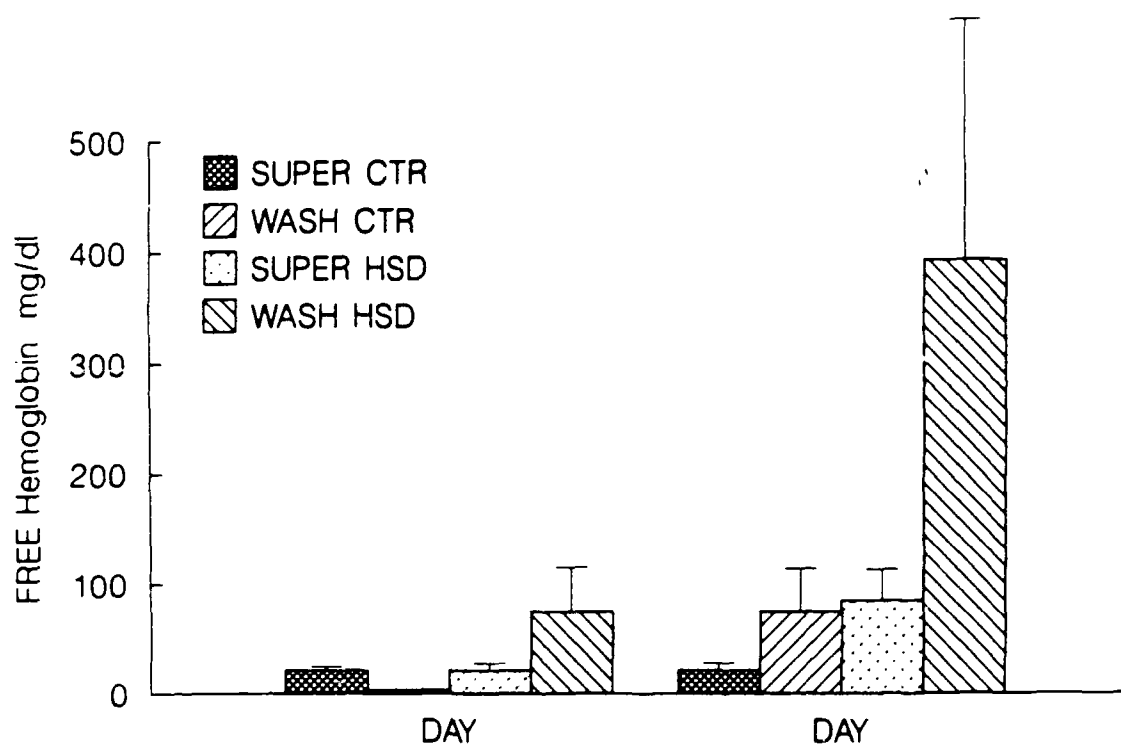


Figure 2: Supernatant hemoglobin from lysed red cells. A concentration of about 150 mg/dl would be equivalent to 1% lysis of the red cells. Error bars are SD.

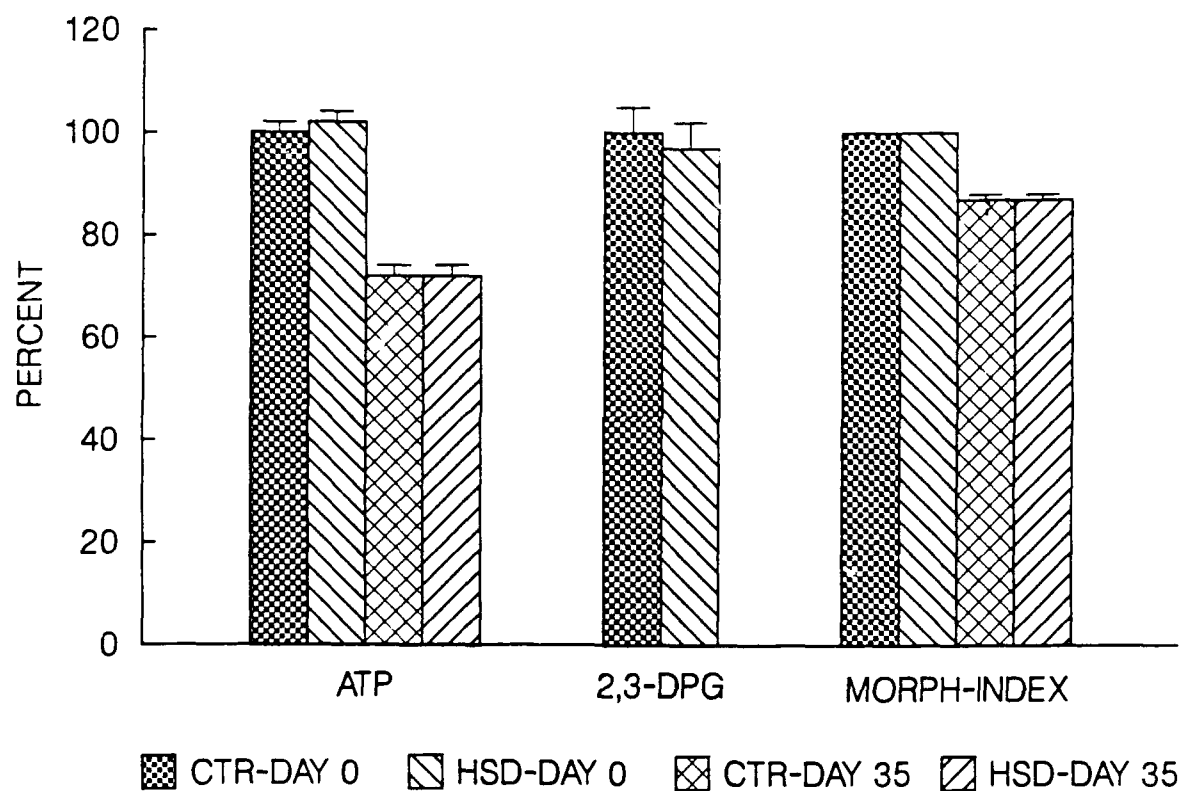


Figure 3: Red cell Morphology Index expressed as percent (100% is all biconcave disks) and red cell ATP and 2,3-DPG expressed as percent of initial values. Day 35 2,3-DPG values are near zero. Error bars are SEM.

Table 1
SUMMARY DATA OF OSMOTIC FRAGILITY CURVES
(MEAN \pm SD)

	CTR-Day0	HSD-Day0	CTR-Day35	HSD-Day35
50% Lysis Point	0.4610 \pm .02	0.4775 \pm .01	0.5083 \pm .02	0.5262 \pm .04
Regres. Slope	881 \pm 215	1041 \pm 156	699 \pm 136	626 \pm 152
Regres. r-value	0.952 \pm .03	0.961 \pm .04	0.974 \pm .02	0.960 \pm .04

Table 2

The Effect of HSD¹ and/or Storage on Red Blood Cell Typing
for ABO Groups and M/N Antigens*

<u>Specimen #</u>	<u>Type</u>	<u>Anti- A</u>	<u>Anti- B</u>	<u>Anti- AB</u>	<u>Anti- M</u>	<u>Anti- N</u>	<u>Anti- D</u>
100	AB+	4+	4+	4+	3+	3+	3+
102	B+	N	4+	4+	3+	3+	3+
103	AB+	2+(3+)	4+	4+	3+	3+	3+
104	B+	N	4+	4+	3+	N	3+
105	A+	4+	N	4+	3+	3+	3+
106	O+	N	N	N	3+	N	3+
107	A+	4+	N	4+	3+	N	3+
108	O+	N	N	N	3+	3+	3+
109	O+	N	N	N	3+	N	3+
110	A+	4+	N	4+	3+	3+	3+
111	B+	N	4+	4+	3+	3+	3+
112	O+	N	N	N	3+	3+	3+

N = No agglutination, Negative

¹HSD = 7.5% Hypertonic Saline in 6% Dextran-70

* = Identical results were obtained following 30 min incubation of normal saline or HSD with fresh blood at a ratio of 1:5 (v/v). Further, repeating the incubations with whole blood stored for 35 days produced identical results as with fresh blood.

Specimen # 103 (3+): Strength of agglutination increased after 35 days in both the control and HSD incubation.

For the above specimens, all Rh and saline controls were negative.

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